

US EPA ARCHIVE DOCUMENT

**Data Evaluation Report on the Acute Toxicity Effects of EF-1521 (AI: Trifluralin) on Earthworms**

PMRA Submission #: {.....}

EPA MRID #: 47807008

**Data Requirement:** PMRA Data Code {.....}  
EPA DP Barcode 367525  
OECD Data Point {.....}  
EPA MRID 47807008  
EPA Guideline 850.6200; OECD 207

**Test material:** EF-1521 (AI: Trifluralin) Purity: 48%  
**Common name**  
**Chemical name:** IUPAC  $\alpha,\alpha,\alpha$ -Trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine  
CAS name 2,6-Dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine  
CAS No. 1582-09-8  
Synonyms Trifluralin 480 g/L

**Primary Reviewer:** Moncie Wright  
Staff Scientist, Cambridge Environmental

**Signature:** *Moncie V Wright*

**Date:** 11/3/09

**Secondary Reviewer:** Teri S. Myers  
Senior Scientist, Cambridge Environmental

**Signature:** *Teri S Myers*

**Date:** 12/07/09

**Primary Reviewer:** Christine Hartless

**Date:** 4/23/10

{EPA/OECD/PMRA} *Christine Hartless*

4-23-10

**Secondary Reviewer(s):** {.....}  
{EPA/OECD/PMRA}

**Date:** {.....}

**Reference/Submission No.** {.....}

**Company Code** {.....} [For PMRA]  
**Active Code** {.....} [For PMRA]  
**EPA PC Code** 036101

**Date Evaluation Completed:** 4/23/10

**CITATION:** Gillham, Angela M. 1999. EF-1521 (Trifluralin 480 EC) Acute Toxicity (LC<sub>50</sub>) to the Earthworm (*Eisenia foetida*). Unpublished study performed by Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory study ID: DOS 044/993592. Study sponsored by Dow AgroSciences, Letcombe Laboratory, Oxfordshire, England. Sponsor study ID: GHE-T-1087. Study completed November 1, 1999.

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## EXECUTIVE SUMMARY:

In an acute toxicity study, earthworms (*Eisenia foetida*) were exposed to EF-1521 (AI: Trifluralin) at nominal concentrations of 0 (negative control), 95, 171, 309, 556, and 1000 mg EF-1521/kg dw soil (artificial substrate). These concentrations were equivalent to 0 (negative control), 46, 82, 148, 267, and 480 mg Trifluralin/kg dw soil. The reference chemical used was chloroacetamide (concentrations not reported), which had an  $LC_{50}$  value of 53.1 mg/kg dw soil.

After 14 days of exposure, mortality was 0% in the negative control and the nominal 46, 82, 148, and 267 mg Trifluralin/kg dw soil treatment groups. Mortality was 15% in the 480 mg ai/kg dw soil treatment group. Percent survival was affected at the 480 mg ai/kg dw soil treatment level, yielding NOAEC and  $LC_{50}$  values of 267 and >480 mg ai/kg dw soil, respectively.

Mean % weight gain was 10% in the negative control, and was 8, 5, -5, -16, and -26% in the nominal 46, 82, 148, 267, and 480 mg Trifluralin/kg dw soil treatment groups, respectively. Body weight change was affected in the three highest test levels, yielding NOAEC and  $EC_{50}$  values of 82 and >480 mg ai/kg dw soil, respectively. The LOAEC value, based on % weight gain, was 148 mg ai/kg dw soil.

Sub-lethal effects were observed for test organisms at the 267 mg ai/kg dw soil treatment level (worms on the sides of the jars). Test organisms in the 480 mg ai/kg dw soil treatment level were observed on the soil surface and on the sides/bottoms of the jars.

This study is scientifically sound and classified as a Supplemental non-guideline study (although it follows OECD 207, EPA does not have a guideline for a 14-day earthworm test).

## **Results Synopsis**

**Test Organism Size/Age(Mean Wt or Length):** 403.8-409.8 mg  
(based on group mean values at study initiation)

### **Mortality**

$LC_{50}$ : ≥480 mg ai/kg dw soil      95% C.I.: N/A  
NOAEC: 267 mg ai/kg dw soil

### **Sublethal (weight gain)**

$IC_{50}$ : ≥480 mg ai/kg dw soil      95% C.I.: N/A  
NOAEC: 82 mg ai/kg dw soil; based on % weight gain

**Endpoint(s) Affected:** Mortality, % weight gain, sublethal effects such as lying on the sides/bottoms of the jars and lying on the soil surface

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## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The study was conducted following OECD Guideline 207 for Testing of Chemicals, "Earthworm, acute toxicity tests" (1984), and EEC Directive 87/302/EEC, Part C, Methods for determination of ecotoxicity, "Toxicity for earthworms: Artificial soil test".

The test was only conducted for 14 days; the reviewer used the guidance for a 28-day sub-chronic test to note deviations in acclimation and environmental conditions. The following deviations from OPPTS Guideline 850.6200 were noted:

1. The % organic carbon content of the soil was not reported.
2. Only the pH of the soil before treatment was reported; OPPTS guidelines suggest that daily pH values be reported.
3. The concentrations of the test mixtures were not analyzed; OPPTS guidelines suggest that test concentrations be measured daily.
4. The study author did not report quarantining and observing the earthworms; OPPTS guidelines suggest this should occur for at least 14 days prior to testing.
5. Acclimation was shorter than recommended 14 days, environmental characteristics during acclimation were not reported.
6. The pretest health of the earthworms was not reported.

These deviations do impact the acceptability of the study.

**COMPLIANCE:** Signed and dated No Data Confidentiality, Quality Assurance and GLP statements were provided. This study was conducted in compliance with U.S. EPA Title 40 Code of Federal Regulations Part 160 (1989), and with OECD principles (EN/MC/CHEM(98)17; 1998).

### A. MATERIALS:

**1. Test Material** EF-1521 (AI: Trifluralin)

**Description:** Dark orange liquid

**Lot No./Batch No. :** C0523-35 (batch no.)

**Purity:** 468 g/L (actual); 480 g/L (nominal); implied purity based on study author's reporting of toxicity values in terms of the formulation and active ingredient (48%)

**Stability of compound under test conditions:** Analytical verification was not performed.  
(OECD recommends water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

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**Storage conditions of test chemicals:**

The test material was stored at room temperature.

## Physicochemical properties of EF-1521 (AI: Trifluralin).

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

## 2. Test organism:

**Species:**

Earthworm (*Eisenia foetida*)

(EPA and OECD recommend *Eisenia fetida andrei* (Bouche). The earthworms should weigh 300-600 mg at the beginning of the test.)

**Age at test initiation:**

Adult

**Weight at study initiation:** 403.8-409.8 mg (based on group mean values at study initiation)

**Source:**

Brickyard Farm, Buckworth Cambridgeshire, UK

## B. STUDY DESIGN:

### 1. Experimental Conditions

a. Range-finding Study: A range-find study was not reported.

#### b. Definitive Study

1. Soil

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Table 1: Physicochemical Properties of Natural Soil

Property	Value	Remarks
		Criteria
For natural soil: Texture: % sand % silt % clay Textural classification  For artificial substrate (provide composition):	Artificial soil used in the definitive tests was comprised of 10% sphagnum peat, 20% Kaolin clay, and 70% industrial sand. Calcium carbonate was added to the soil mixture to obtain a pH of 5.5. The initial soil moisture content before treatment was not reported. The % organic carbon was not reported.	<i>Recommended testing medium is artificial soil consisting of a mixture of 68% of No. 70 mesh silica sand, 20% kaolin clay, 10 sphagnum peat moss, and 2% calcium carbonate, mixed and moistened to 35% by weight with deionized/distilled water.</i>
pH (___:___ soil:water)	N/A	
Organic carbon (%)	N/A	
Moisture (%)	N/A	

Table 2: Experimental Design

Parameter	Detail	Remarks
		Criteria
Acclimation:  duration: conditions (state if same as the test conditions): health:	None reported; refer to adjacent Remarks N/A N/A Not reported	The study author reported in the protocol that the worms will be acclimated to the soil for at least 24 hours.  Worms were received on 14 and 19 May 1999; test initiated on 21 May 1999. No other details of acclimation provided.  <i>Earthworms should be acclimated at test temperature for 7 days.</i>
Soil [fresh or stored]	Freshly prepared	
Test Container  material size	Glass 1 L	

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Parameter	Detail	Remarks
		Criteria
amount of soil/substrate	Average of 740 g wet weight	
No. of replicates:		
per treatment group:	4	<i>Recommended number of replicates include at least 3 and a control.</i>
per control:	4	
No. of earthworms per treatment	40 total for each treatment group and the control	
		<i>Recommended number of earthworms per treatment include a minimum of 30 plus a control; 10 per each of three replicates and a control.</i>
Solvents used or not (if yes report the name and concentration)	N/A	
Rates of application:		
Nominal (formulation):	0 (negative control), 95, 171, 309, 556, and 1000 mg EF-1521/kg dw soil	<i>Earthworms should be exposed to at least five test concentrations, in geometric series, in which the ratio is between 1.5 and 2.0 mg of test chemical per kg (air-dry weight) of artificial soil.</i>
Nominal (active ingredient):	0 (negative control), 46, 82, 148, 267, and 480 mg Trifluralin/kg dw soil	
Measured:	N/A	
Reference chemical (if used) name:	Chloroacetamide	
concentration:	Not reported	
Test conditions:		

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Parameter	Detail	Remarks	
		Criteria	
temperature Lighting conditions Moisture	20-22°C Continuous 400 lux Moisture was 35% of the dry weight at test initiation. After 14 days, moisture content was 32% of the soil dry weight.	Recommended temperature: 22 ± 2°C Recommended lighting: Continuous illumination, with a light intensity of 400 lux Recommended relative humidity: above 85%	
Duration of the study	14 days	Recommended duration of study is 28 days.	

## 2. Observations:

Table 3: Observations

Parameters	Details	Remarks	
		Criteria	
Observation intervals	Mortality and weights of the worms as replicate and group mean weights of worms were assessed on days 7 and 14. Behavioral abnormalities and pathological signs were observed daily.	Recommended observation intervals are days 7, 14, 21, and 28.	
Parameters measured including the sublethal effects/toxicity symptoms	-Mortality -Weight of surviving earthworms (weight at days 0, 7, and 14) -Sub-Lethal Effects (behavior and pathological signs)	The test is usually not acceptable if more than 20% of control earthworms die or the total mean weight of control earthworms lose 20% or more of body weight.	
Were raw data included?	Replicate data for mortality and average weight was included. Data for individual worms not included.		
Other observations, if any	None		



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## II. RESULTS AND DISCUSSIONS

### A. MORTALITY:

After 14 days of exposure, mortality was 0% in the negative control and the nominal 46, 82, 148, and 267 mg Trifluralin/kg dw soil treatment groups. Mortality was 15% in the 480 mg ai/kg dw soil treatment group. The study author reported a 14-day  $LC_{50}$  value of >1000 mg EF-1521/kg dw soil, which was equivalent to >480 mg ai/kg dw soil.

Table 4: Effect of EF-1521 (AI: Trifluralin) on Mortality of *Eisenia foetida*

Nominal Concentrations (mg ai/kg soil)	Observation period			
	Day 7		Day 14	
	No Dead	% mortality	No Dead	% mortality
Control	0	0	0	0
46	0	0	0	0
82	0	0	0	0
148	0	0	0	0
267	0	0	0	0
480	6	15	6	15
NOAEC	Not reported			
LOAEC	Not reported			
$LC_{50}$	>1000 mg EF-1521/kg dw soil, equivalent to >480 mg ai/kg dw soil			
Reference chemical % mortality: $LC_{50}$ (95% confidence limits)	53.8 (48.7-60.0) mg/kg		53.1 mg/kg (48.1-59.3) mg/kg	

### B. SUB-LETHAL TOXICITY ENDPOINTS:

The study author reported replicate weights and treatment weights (means) for days 0, 7, and 14. From day 0 to 7, weight loss was observed in the 267 and 480 mg Trifluralin/kg dw soil treatment groups. From day 7 to 14, weight loss was observed in all treatment groups, including the control. The NOAEC value was reported as being 171 mg EF-1521/kg dw soil, which was equivalent to 82 mg ai/kg dw soil.

In addition, test organisms at the 267 mg ai/kg dw soil treatment level were observed on the sides of the jars. Test organisms in the 480 mg ai/kg dw soil treatment level were observed on the soil surface and on the sides/bottoms of the jars.

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**Table 5: Sub-lethal Effect of EF-1521 (AI: Trifluralin) on *Eisenia foetida*. Average weights were used**

Nominal Concentration s (mg ai/kg soil)	Observation period						
	Day 0		Day 7		Day 14		
	weight	% loss	weight	%gain (day 0 to day 7)	weight	%gain (day 7 to day 14)	%gain (day 0 to day 14)
Control	410	N/A	459	12	450	-2	10
46	410	N/A	445	9	443	0	8
82	405	N/A	439	8	424	-3	5
148	406	N/A	408	0	385	-6	-5
267	410	N/A	381	-7	345	-9	-16
480	404	N/A	324	-20	299	-8	-26
NOAEC	171 mg EF-1521/kg dw soil, equivalent to 82 mg ai/kg dw soil						
LOAEC	Not reported						
EC <sub>50</sub>	Not reported						

## **C. REPORTED STATISTICS:**

Mortality was visually determined due to only a 15% effect at the highest test level. A NOAEC was not identified by the study author.

Mean body weight per replicate data from days 7 and 14 were analyzed using a one-way ANOVA, with the mean bodyweight at day 0 included as a covariate as this improved precision. Williams' test was then conducted. Analyses were performed using Genstat 5.3.2. For both endpoints (average weight at day 7 and average weight at day 14), the NOAEC was identified as 82 mg ai/kg dw soil.

## **D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Mortality did not reach 50%, therefore the LC<sub>50</sub> value was visually determined as greater than the highest test concentration (≥480 mg ai/kg dw soil). The NOAEC based on mortality was visually determined at 267 mg ai/kg dw soil.

The reduction in weight did not reach 50%; therefore, the IC<sub>50</sub> value was also visually determined as greater than the highest test concentration (≥480 mg ai/kg dw soil). The reviewer tested the percent weight gain data (from day 0 to day 14) for normality using the Chi-square and Shapiro-Wilks tests and for homogeneity of variance using the Hartley and Bartlett's tests via Toxstat 3.5 statistical software. The data met the assumptions of ANOVA, thus the NOAEC value was determined using the parametric Dunnett's and Williams' tests via Toxstat statistical software. All analyses were conducted using the nominal concentrations.

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### Mortality

LC<sub>50</sub>: >480 mg ai/kg dw soil 95% C.I.: N/A

NOAEC: 267 mg ai/kg dw soil

### Sublethal (weight gain)

IC<sub>50</sub>: >480 mg ai/kg dw soil 95% C.I.: N/A

NOAEC: 82 mg ai/kg dw soil; based on % weight gain

Endpoint(s) Affected: Mortality and % weight gain

### E. STUDY DEFICIENCIES:

- The study was conducted for 14 days, OPPTS guidelines recommend 28 days.
- The % organic carbon content of the soil was not reported.
- Only the pH of the soil before treatment was reported; OPPTS guidelines suggest that daily pH values be reported.
- The concentrations of the test mixtures were not analyzed; OPPTS guidelines suggest that test concentrations be measured daily.
- The study author did not report quarantining and observing the earthworms; OPPTS guidelines suggest this should occur for at least 14 days prior to testing.
- Acclimation was shorter than recommended 14 days, environmental characteristics during acclimation were not reported.
- The pretest health of the earthworms was not reported.

### F. REVIEWER'S COMMENTS:

The reviewer's and study author's results were in agreement with regard to the LC<sub>50</sub> and NOAEC value for % weight gain. The reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

No OPPTS guidance exists for a 14-day acute earthworm toxicity test, thereby making this a non-guideline test. Therefore, the reviewer used the guidance for a 28-day sub-chronic test to note deviations in acclimation and environmental conditions.

The experiment was conducted from July 21 to August 4, 1999.

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### G. CONCLUSIONS:

This study is scientifically sound and classified as a Supplemental non-guideline study (although it follows OECD 207, EPA does not have a guideline for a 14-day earthworm test).

Percent survival was affected at the 480 mg ai/kg dw soil treatment level, yielding NOAEC and LC<sub>50</sub> values of 267 and ≥480 mg ai/kg dw soil, respectively. Body weight change was affected in the three highest test levels, yielding NOAEC and IC<sub>50</sub> values of 82 and ≥480 mg ai/kg dw soil, respectively.

#### Mortality

LC<sub>50</sub>: ≥480 mg ai/kg dw soil      95% C.I.: N/A

NOAEC: 267 mg ai/kg dw soil

#### Sublethal (weight gain)

IC<sub>50</sub>: ≥480 mg ai/kg dw soil      95% C.I.: N/A

NOAEC: 82 mg ai/kg dw soil; based on % weight gain

**Endpoint(s) Affected:** Mortality, % weight gain, sublethal effects such as lying on the sides/bottoms of the jars and lying on the soil surface

### III. REFERENCES:

Berkson, J. (1944) Application of the logistic function to bio-assay. *J. Amer. Statist. Assoc.* **39**, 357-365.

Williams, D.A. (1986) Interval Estimation of the median Lethal Dose. *Biometrics*, **42**, 641-645.

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**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

Trifluralin & earthworms 14-day % weight gain; mg ai/kg

File: 7008w Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	9	8	7	0

Calculated Chi-Square goodness of fit test statistic = 5.3637

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Trifluralin & earthworms 14-day % weight gain; mg ai/kg

File: 7008w Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 367.500

W = 0.973

Critical W (P = 0.05) (n = 24) = 0.916

Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

Trifluralin & earthworms 14-day % weight gain; mg ai/kg

File: 7008w Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 5.50

Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3

Actual values ==> R (# groups) = 6, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

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Trifluralin & earthworms 14-day % weight gain; mg ai/kg  
File: 7008w Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

-----  
Calculated B statistic = 2.79  
Table Chi-square value = 15.09 (alpha = 0.01)  
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00  
Used for Chi-square table value ==> df (#groups-1) = 5  
-----

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Title: Trifluralin & earthworms 14-day % weight gain; mg ai/kg  
File: 08WORM-1. Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	4180.3333	836.0667	40.9502
Within (Error)	18	367.5000	20.4167	
Total	23	4547.8333		

(p-value = 0.0000)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Trifluralin & earthworms 14-day % weight gain; mg ai/kg  
File: 08WORM-1. Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control&lt;Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Neg control	10.0000	10.0000		
2	46	8.2500	8.2500	0.5477	
3	82	5.0000	5.0000	1.5649	
4	148	-5.0000	-5.0000	4.6948	*
5	267	-16.0000	-16.0000	8.1376	*
6	480	-25.7500	-25.7500	11.1892	*

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

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Title: Trifluralin & earthworms 14-day % weight gain; mg ai/kg  
File: 08WORM~1. Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2			Ho: Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	4			
2	46	4	7.7001	77.0	1.7500
3	82	4	7.7001	77.0	5.0000
4	148	4	7.7001	77.0	15.0000
5	267	4	7.7001	77.0	26.0000
6	480	4	7.7001	77.0	35.7500

Title: Trifluralin & earthworms 14-day % weight gain; mg ai/kg  
File: 08WORM~1. Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2			Ho: Control<Treatment		
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	4	10.0000	10.0000	10.0000
2	46	4	8.2500	8.2500	8.2500
3	82	4	5.0000	5.0000	5.0000
4	148	4	-5.0000	-5.0000	-5.0000
5	267	4	-16.0000	-16.0000	-16.0000
6	480	4	-25.7500	-25.7500	-25.7500

Title: Trifluralin & earthworms 14-day % weight gain; mg ai/kg  
File: 08WORM~1. Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2			Ho: Control<Treatment		
IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg control	10.0000				
46	8.2500	0.5477		1.7300	k= 1, v=18
82	5.0000	1.5649		1.8200	k= 2, v=18
148	-5.0000	4.6948	*	1.8500	k= 3, v=18
267	-16.0000	8.1376	*	1.8600	k= 4, v=18
480	-25.7500	11.1892	*	1.8700	k= 5, v=18

s = 4.5185